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BIOFABRICATION OF GOLD NANOPARTICLES USING FRESH WATER GREEN ALGAE CHARA VULGARIS AND ITS CHARACTERIZATION STUDIES

P.Mohanapriya[1*], A.B.Suma[2] and R.Sathiyapriya[3]

[1*] Department of Nanoscience and Technology,
GKM College of Engineering and Technology
Chennai – 600 063

[2] Department of Biomedical Engineering
GKM College of Engineering and Technology
Chennai – 600 063

[3] Department of Chemistry,
GKM College of Engineering and Technology
Chennai – 600 063

RESEARCH ABSTRACT

Bio-synthesis of nanoparticles is gaining huge importance, these days. Gold nanoparticles (GNPs) are widely used for various applications such as drug delivery, bioprobe, Imaging and drug targeting. Various sources such as plant extracts, fungi and microbes are used for the synthesis of GNPs. Algae are living in a sessile aquatic environment for its survival and it has certain bioactive compounds such as carbohydrates, aminoacids, proteins, fatty acids, flavonoids, antioxidants, saponins, etc. However, there are only limited studies for the synthesis of GNPs using algae. Hence, in this study, clinically potent GNPs were synthesized using fresh water green algae *Charavulgaris*. In the present study, the colour of the prepared solution changes from yellowish green to red wine indicates the synthesis of gold nanoparticles. In addition, UV-Vis spectrophotometer, SEM, XRD and FT-IR analysis was done for the analyses of gold nanoparticles. Furthermore, antibacterial study was done and it showed maximum sensitivity to the given bacterial species.

Key words: Bio synthesis, gold nanoparticles, fresh water algae, Charavulgaris, anti-microbial study

I. INTRODUCTION

Nowadays, nanoparticles are gaining huge interest in the research field because the properties of the particles change when its size is decreased to nanometres (Raliya and Tarafdar 2012). Thus, they exhibit distinct properties which are different from that of its bulk form. For instance, gold nanoparticles possess distinct semiconductance and magnetic properties rather than its lustrous and shiny finish (Pillai et al. 2017). Therefore, these unique properties of gold as a nanomaterial open up a new door in the field of nanotechnology, especially for biomedical applications.

Nanomedicine involves the implication of nanotechnology in the field of medicine.Gold nanoparticles (GNPs) owing to its properties such as nontoxicity, rigidity and chemical stability, bioinertness, biodegradability, tunable functionality, optical properties and cellular imaging abilityare increasingly gaining attention in the field of nanomedicine (Chulz-Dobrick, Sarathy&Jansen 2005; Leonard et al. 2011). Hence, GNPs are applied in the various fields of nanomedicine such as drug delivery, bioprobe, Imaging and drug targeting. In addition, GNPs are also employed in the field of separation sciences and electrochemistry.

The property of gold nanoparticle is related to its synthesis process. The synthesis step determines not only its shape and size of the nanoparticles but also its electronic, catalytic and optical properties. This signifies the role of specific synthesis method for the production of GNPs.

A number of chemical, physical, biological and hybrid synthesis methods are available for the production of GNPs. Chemical synthetic methods of GNPs involve the use of chemicals such as sodium borohydride, sodium citrate and organic solvents. The use of these chemicals is greatly limited owing to their environmental toxicity and health hazardsespecially, biomedical applications (Cliffel, Turner & Huffman 2008; Sperling et al. 2008) .The major disadvantage of physical and chemicalsynthesis methods of GNPs is that they are highly costly and also posesharmful environmental and biological hazards (Li 2011). This clearly indicates that there is a need for the development of a sustainable, non-toxic and cost-effective method for the synthesis of GNPs, in particular, clinical applications.

From the previous study,it is clear that gold nanoparticles are synthesised from various sources such plant extracts (Nath&Banerjee 2013), fungi and microbes (Sastry et al. 2003).Fakhri et al. (2014) indicated the importance of aquatic resources in the treatment of cancer and pathogenic infection. One of the important aquatic multicellular plant-like organism is algae, which are found in fresh water, moist soil, salt water,and rock surfaces. They are major producers of minerals, fatty acids, vitamins, and proteins etc (Pulz& Gross 2004). Algae are commonly used in pharmaceutical (as antioxidants, virostatic and antibiotic agents), food and cosmetic industry. Due to the wide range of applications of algae, studies focussing on the biosynthesis of GNPs using algae are increasing. A number of studies focussed on marine algae for the green synthesis of GNPs (Azizi et al. 2013; Kumar et al. 2013; Prasad et al. 2013). Nonetheless, there are only limited studies using fresh water algae for the synthesis of GNPs. One of the major fresh water algae is Chlorophyta or Charophyta (green algae).*Characeae*is otherwise known as stoneworts. It is a green algae

found in fresh water belonging to the class of Charophyceae. They appear as whorls of branches at their tissue node.

Synthesis of metallic nanoparticles using marine algae has been extensively studied for their antimicrobial properties (Azizi et al. 2013; Kumar et al. 2013; Prasad et al. 2013). However, reports on fresh water algae are lacking. Hence, the major aim of this study is to develop an eco-friendly, green approach for the synthesis of gold nanoparticles. In this study, green algae extract is used as a reducing agent for the biosynthesis of GNPS. The synthesized nanoparticles were confirmed using SEM analysis. Moreover, the antibacterial activity of the synthesized gold particles was also performed. Thus, the present study explores the possibilities of employing this alga (*Charavulgaris*) in pharmaceuticals for its antibacterial activity.Moreover, this is the first study to synthesiseGNPs using the fresh water green alga(*Characeae*).

II. MATERIALS AND METHODS

A. *Reagents and materials*

Chemicals:

Analytical chemical chloroauric acid and deionised water is used for the synthesis of gold nanoparticles. Hydrogen peroxide solution is used for the antioxidant study. The chemicasl were obtained from Loba chemical Pvt limited (Chennai, T. N, India).

B. Collection of algae:

The Fresh water green algae *(Chara sp*.) were collected from the wells of Kanchipuram District located in Tamil Nadu. The Samples were collected in sterile plastic bags and taken to the laboratory under normal condition. The samples were initially rinsed thoroughly with fresh water to remove sand and dust particles attached to it and finally washed with distilled water. The water was removed by using filter paper. The algal species were identified based on their morphological characters under microscopic image. The algae were dried for 3weeks to remove moisture content and stored for further process.

C. Preparation of algae extract:

The dried algae (*Chara sp*.) were weighed (5g) and grounded into powder form with the help of blender. Then 50mL of double distilled water was added to the powdered algae and boiled at 70- 80^{o}C for 15- 20 minutes to extract crude solution. After boiling, the crude solution was filtered using Whatman filter paper No:1 and stored under the frozen condition for the further investigation.

D. Biosynthesis of gold nanoparticles:

The Chloroauric solution was prepared by dissolving 1mg of $HAuCl_4$ in 100mL of deionised water. 10mL of algae extract was added to 90mL of 1mMChloroauric solution by continuous stirring and kept at room temperature for 24 hours. A colour change from yellowish green to purplish red was observed which indicates the bioreduction of gold ions to nanoparticles. The colour change of the solution was observed

under the UV- Visible spectrometer at wavelength range from 200 to 700nm. This indicates the reduction of the Au+ ions andbiosynthesis of goldnanoparticles.

E. Confirmation by SEM, XRD and FT- IR analysis:

The bio reduced solution containing Au nanoparticles was centrifuged at 2000rpm for 20minutes. After centrifugation, the pellet was obtained and dried at hot plate. The powdered form of nanoparticles were collected and analyzed under the SEM, XRD and FT-IR analysis..

F. Antibacterial activity:

Antibacterial activity was determined using well diffusion technique in Muller Hinton agar. Briefly, the prepared media was poured on the sterile petri plate and wells were made by usingsterile well puncher and the plate was inoculated with *E. coli*of freshly prepared culture. Various concentrations of gold nanoparticles solution such as 10µg/ml, 50 µg/ml, and 100 µg/ml were loaded into the labelled wells. Then, the plates were incubated at 37°C for 24 hours and the zones of inhibition formed around the wells were measured (in mm diameter).

III. RESULTS AND DISCUSSION

In the present study, GNPs were synthesized using fresh water green algae (*Chara sp.*)to 1mM $HAuCl_4$ solution. The red wine colour indicates the presence of synthesis GNPs and it was confirmed by UV-Visible spectrophotometer characterization. The colorchange is due to changes in oxidationstate of Gold metal and reduction of chloroaurate ions.In our study, gold nanoparticles underwent reduction by a few unidentified biomolecules in *Chara sp.*(Fujiwaraet al. 2007). The peak obtained at 540.1nm which confirms the GNPs were synthesized.

FIGURE 1:*Charavulgaris* synthesised gold nano particles

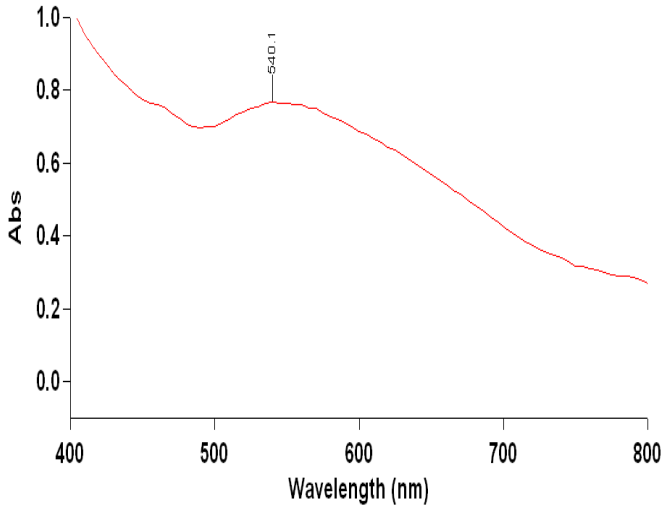

FIGURE 2: UV Spectrum of *Charavulgaris* synthesised gold

A. Characterization Studies

Figure 2 illustrates SEM image of 1 mM $HAuCl_4$ solution inoculated with *Chara sp.* extract at room temperature. From fig. (3), it is clear that sphere shaped nanoparticles were formed ranging from 40nm to 51.47 nm. Similar results were obtained in the study of Daisy and Saipriya (2012)who reported the

synthesis of gold nanoparticles by using the Cassia fistula. In that study, the SEM images exhibited different ranges of gold nanoparticles such as rectangle, square and triangle from the bark extract of *C. fistula*.

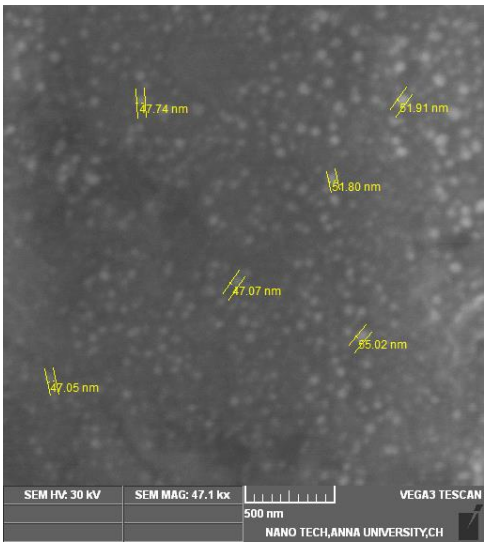

FIGURE 3: SEM image of *Charavulgaris* synthesised gold nanoparticles

Figure 4 illustrates the XRD spectrum of synthesized gold nanoparticles using *Charavulgaris*, it shows the synthesized gold nanoparticles were found to be Face centred cubic crystal the peaks found at 70, 74 and 69 with 2 theta values such as 37.88, 46.24 and 64.52 respectively.

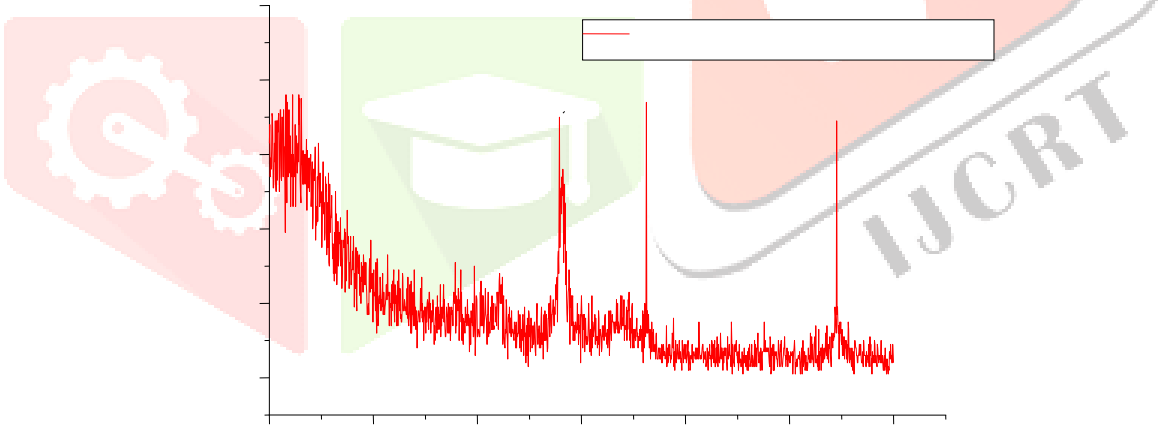

FIGURE 4: XRD spectrum of *Charavulgaris* synthesised gold nanoparticles

Figure: 5 illustrate the FT-IR spectrum of synthesised *Charavulgaris* synthesised gold nanoparticles showed that the presence of Alcohol/ Phenol O-H Stretch, Alkyl C-H Stretch, Carboxylic acid –OH stretch, Alkenyl C=C stretch, Aromatic C=C bending, Nitro compound -NO_2, C-S disulphide and S-S(Polysulphide) Stretching groups.

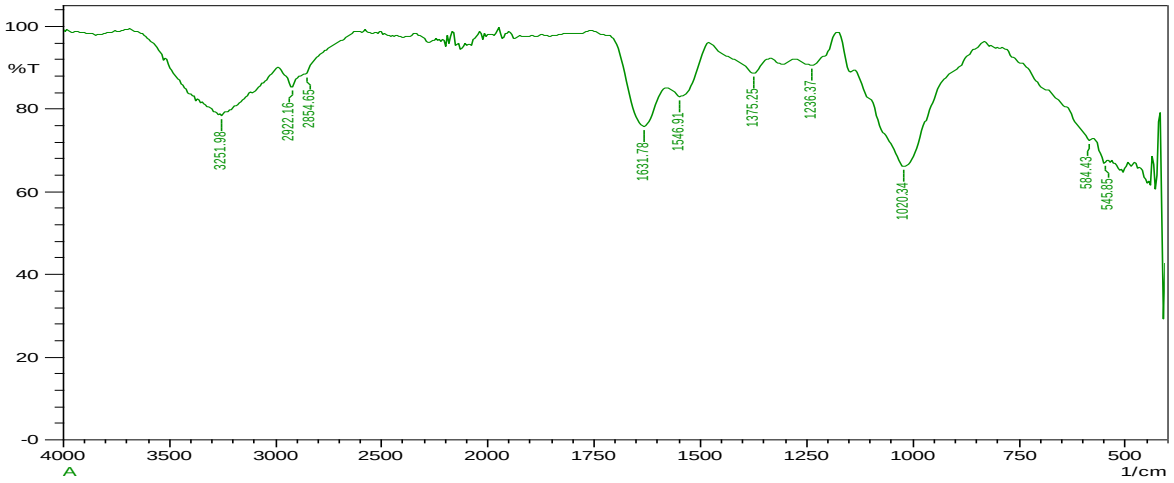

FIGURE 5: FT-IR spectrum of *Charavulgaris* synthesised gold nanoparticles

B. **Antibacterial activity**:

In this study *Escherichia coli* and *Escherichia coli* DH3 were employed to study the antibacterial activity of GNPsat 4 different concentrations i.e., 10µg/mL, 50 µg/mL, 100 µg/ml and 150 µg/ml. Out of the 3 different concentrations, 150 µg/ml hasgot maximum inhibition zone of 12 mm and 10 mm for *Escherichia coli* and *Escherichia coli* DH3, respectively. The least range of inhibition (4 mm& 6 mm) was found in 10µg/ml for *Escherichia coli* and *Escherichia coli* DH3,respectively. From this study, it is clear thatthe area of inhibition zones increased with increasing order of gold nanoparticles concentration. Thus, GNPs concentration is directly proportional to the area of inhibition zone. The results of the present study are inline with the results of Kannan et al. (2013) in which marine algae*Turbinariaconoides*was employed to synthesise GNPs. In that study,inhibition zone area increased with increasing concentration of GNPs.

FIGURE 6: Antimicrobial activity of*Charavulgaris* synthesised gold nanoparticles against *E.coli*

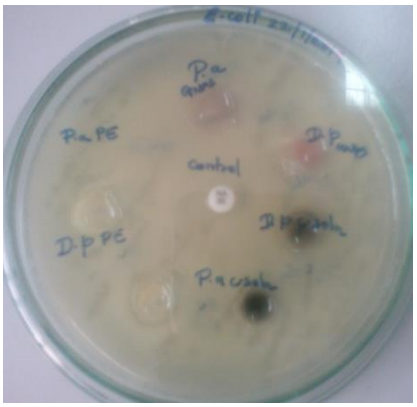

FIGURE 7: Antimicrobial activity of*Charavulgaris* synthesised gold nanoparticles against *E.coli*DH3

IV. FUTURE RECOMMENDATIONS

Future studies can focus on the effect of parameters such as time or pH in order to control the size and shape of the metallic nanoparticles.The present study method can beextended for the synthesis of other metal nanoparticles and can be used for a range of catalysis reactions.

V. CONCLUSION

This is the first study to employ fresh water green algae *Charavulgaris* as a reducing agent in the synthesis of Gold nanoparticles using green chemistry method. The synthesized GNPs was characterised using UV-vis spectrometer and SEM. The SEM results showed spherical shaped GNPs in the range of 40nm to 51.47 nm. The XRD spectrum reveals the synthesised gold nanoparticles found to be FCC crystal. The FT-IR spectrum showed the presence of various functional groups such asAlcohol/ Phenol O-H Stretch, Alkyl C-H Stretch, Carboxylic acid –OH stretch, Alkenyl C=C stretch, Aromatic C=C bending, Nitro compound - NO_2 , C-S disulphide and S-S(Polysulphide) Stretching groups. In addition, it was found out that the synthesized GNPs displayed strong antibacterial activity against*E.Coli and E.Coli DH3 from their zone of inhibition.* With the increasing demand for new sources of antimicrobials, our newly synthesised GNPS using fresh water green algal extracts holds a promising future in the field of nanomedicine against multidrug resistant pathogenic bacteria. However, it is necessary to understand the mechanism of action of GNPS before their application in nanomedicine.

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